Supplementary Materials

Fig. S1. Comparison of parental and 6His-SUMO2^{T90K} expressing HEK293 cells

Fig. S2. Comparison of SUMO2 and SUMO2^{T90K} in conjugation and deconjugation reactions with various substrates.

Fig. S3. Overlap of SUMO2^{T90K} sites in replicate experiments.

Fig. S4. Overlap of sumoylated proteins with published protein level proteomic studies.

Table S1. Sumoylation sites identified in SUMO2^{T90K} expressing cells.

Table S2. diGly modified peptides identified in SUMO2^{T90K} expressing cells.

Table S3. Functional annotation and network analysis of SUMO2^{T90K} expressing cells.

SMfile1.PDF. MS2 spectra of multiply sumoylated peptides identified in SUMO2^{T90K} expressing cells.

SMfile2.PDF. MS2 spectra of branched sumoylated peptides identified from SUMO2 expressing cells.

SUMO1.FASTA. SUMO1 virtual branched peptide database.

SUMO2.FASTA. SUMO2 virtual branched peptide database.

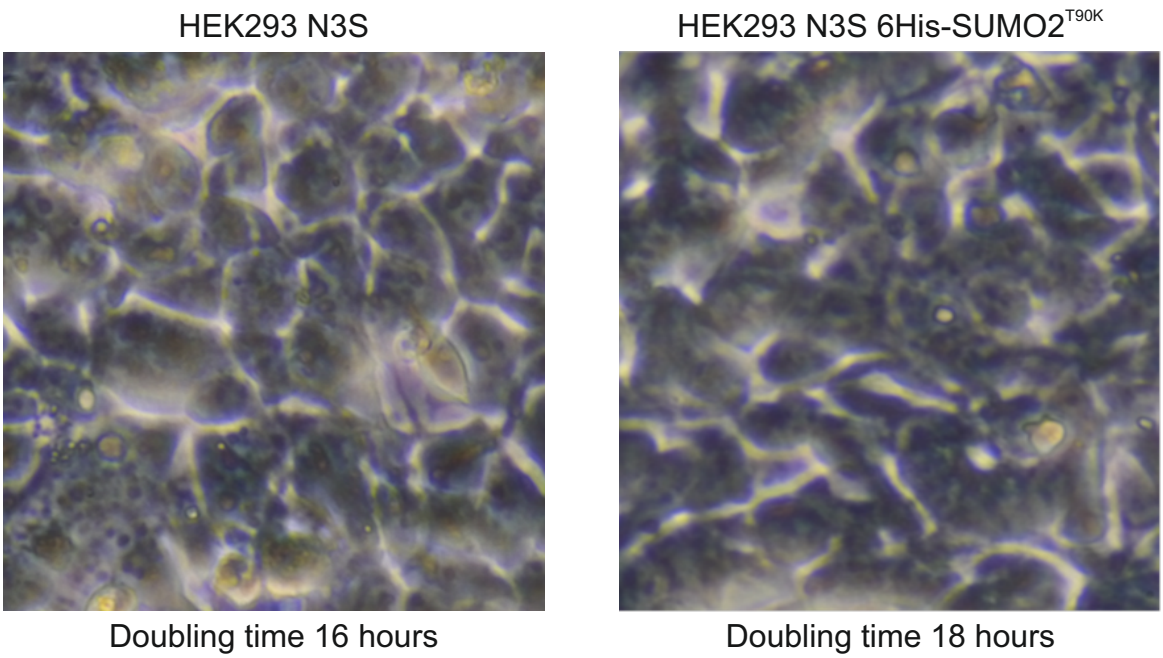

Figure S1. Comparison of parental and 6His-SUMO2^{T90K} expressing HEK293 cells. Bright field images of parental (left) and 6His-SUMO2^{T90K} expressing (right) HEK293 NS3 cells.

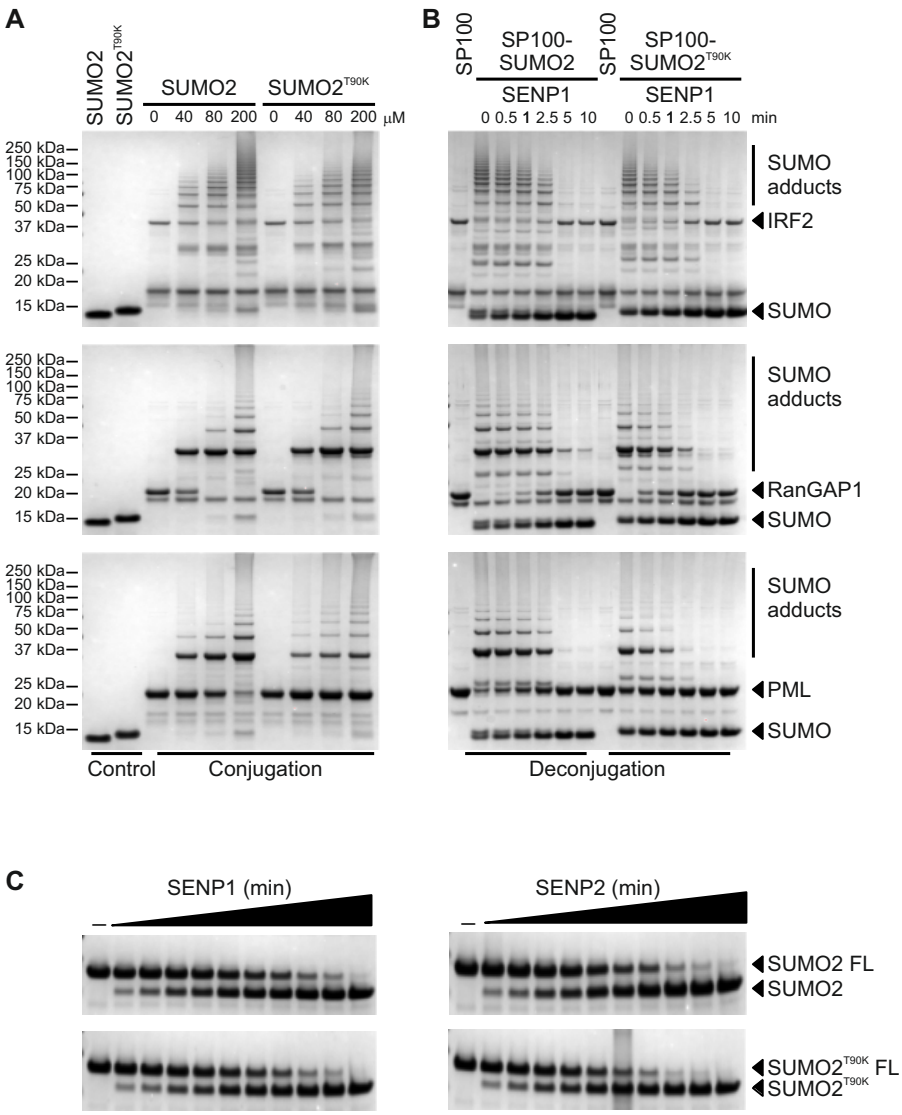

Figure S2. Comparison of SUMO2 and SUMO2^{T90K} in conjugation and deconjugation reactions with various substrates. (A-C) Coomassie-stained protein gels of in vitro enzyme reactions with wild-type and T90K mutant SUMO2 showing conjugation (A) and deconjugation (B) of the indicated substrate proteins (IRF2, RanGAP1, and PML) or processing (C) of the pro-form (FL) of SUMO2.

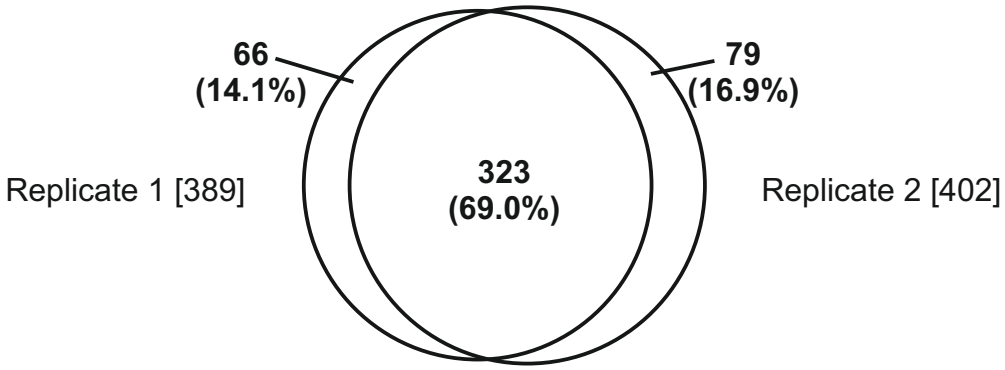

Figure S3. Overlap of SUMO2^{T90K} sites in replicate experiments. Venn diagram showing the overlap between replicate purifications of SUMO2^{T90K} modified peptides. The numbers in brackets show the total number of sites identified in each sample.

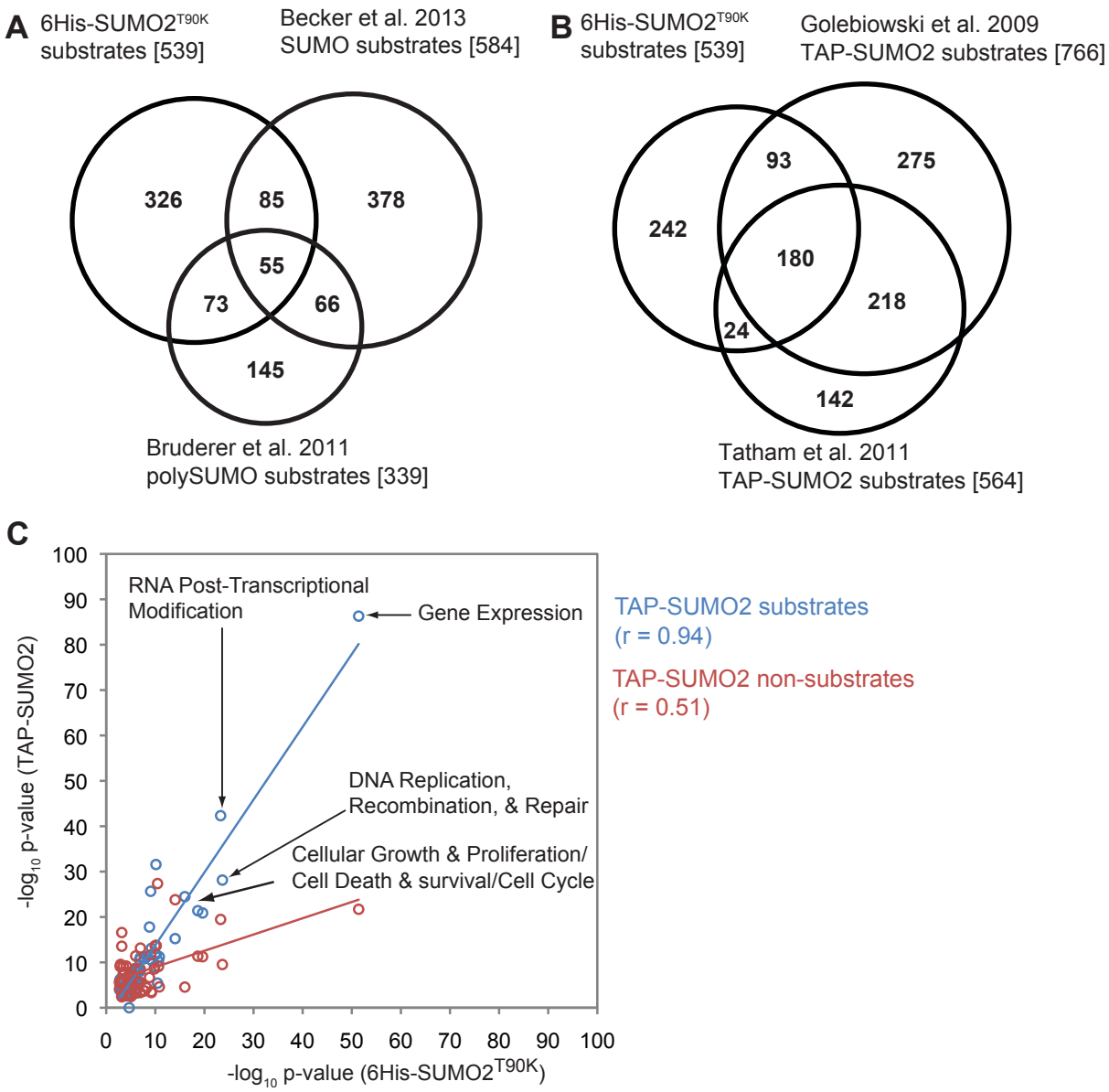

Figure S4. Overlap of sumoylated proteins with published protein level proteomic studies. (A-B) Venn diagram showing the overlap between SUMO^{T90K} modified proteins and (A) proteins found to be modified by endogenous SUMO in Becker *et al.* (21) or by endogenous polySUMO chains after heat shock in Bruderer *et al.* (22) or (B) proteins modified by TAP-SUMO2 in Golebiowski *et al.* (4) after heat shock or by TAP-SUMO2 after proteosomal inhibition in Tatham *et al.* (6). The numbers in brackets indicate the total number of sumoylated proteins. (C) Graph of the results from enrichment analysis of protein function and disease annotations using Ingenuity pathways analysis. Data compare the 539 SUMO2^{T90K} modified proteins with 963 proteins identified as putative TAP-SUMO2 substrates (blue) and 835 proteins that were not sumoylated (red) from Golebiowski *et al.* (4) and Tatham *et al.* (6). Categories of functionally enriched proteins discovered using SUMO2^{T90K} correlated with those for TAP-SUMO2 modified proteins but not non-sumoylated proteins discovered in the same study. Statistical analysis was performed using a Pearson correlation.